Evaluation of Angelicae Radix (Touki) by the Inhibitory Effect on Platelet Aggregation¹⁾

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Quality evaluation of Angelicae Radix (Touki) was discussed referring to its inhibitory effect on platelet aggregation. No better correlation was observed in quality determined morphologically from the above-ground part of Touki and that determined by anti-platelet aggregation (APA) effects. Phthalides and adenosine, known to be the major components of Touki, were found not sufficient to evaluate the Touki quality as regards APA. Other APA active components have been studied and it was concluded that the APA effect of Touki results from the combined action of plural components. The importance of determining quality based on the action of total extract to meet the purpose for which the substance is to be used was suggested.

Keywords Angelicae Radix; *Angelica acutiloba*; evaluation; anti-platelet aggregation; morphological variety; habitat; phthalide; adenosine

Angelicae Radix (Touki) is one of the crude drugs applied most frequently in Sino-Japanese therapy. The commercial preparation is from the dried root of the Angelicae plant which differs by habitat and species: *Angelica acutiloba* KITAGAWA (Obuka Touki) and *A. acutiloba* var. *sugiyamae* HIKINO (Hokkai Touki) in Japan, and *A. sinensis* (OLIV.) DIELS (Kara Touki) in China. The result of any evaluation depends mainly on the habitat or external appearance as judged by experts, who require severe cultivation conditions. Especially, self-pollination is not adapted for the propagation of Angelicae plants, which have highly genetic inhomogeneity, and it is thus very difficult to supply the market with Angelicae Radix of a uniform and stable quality. It is fundamentally desirable to evaluate the crude drugs from their clinical aspects, although to date yield of the major component or total volume of the extracts have been the factors primarily used. Quantitative analyses of the dried material extract and several specific constituents, such as phthalides, nicotinic acid, Vitamine B₁₂, niacin, biotin, choline *etc.*, have been reported,^{2–7)} but no evaluative study of the real therapeutic purpose has been reported. Among the various beneficial effects of Angelicae Radix, its use as a homeostatic remedy for women's disorders has empirically been known.⁸⁾ From the view point of this clinical effect, we report here an evaluation of the anti-platelet aggregation (APA) effect with certain varieties of Angelicae Radix; this is considered to be one of the important factors in improvement of the 'Oketsu' syndrome, which is regarded as a kind of peripheral circulatory disorder and clinically recognized as blood stagnation.

Experimental

The following instruments were used: gas chromatography (GC), Shimadzu GC-9A; high performance liquid chromatography (HPLC), Shimadzu LC-6A; Platelet aggregation tester-counter: TOA Medical Electron Co., Ltd., PL-100, Aggregometer: NKK Hematracer I, Niko Bio-Science Inc., Tokyo.

Plant Material *Angelica acutiloba* KITAGAWA cultivated in Sibukawa, Fujimi-mura and Azumagun-Rokugomura, Gunma Prefecture (Japan) were offered by Mr. T. Hashimoto. A commercial product was purchased from Tochimoto Tenkaido Co., Ltd., Osaka, and morphologically different varieties cultivated in the same place were obtained from Toyama Medicinal Plant Instructive Center and the herbal garden of this university. *A. acutiloba* var. *sugiyamae* HIKINO cultivated in Nayoro, Hokkaido, was offered by Mr. Y. Hatakeyama. All materials except the commercial one were used after treatment with hot water (60 °C, 30 min)

(so-called yu-doshi).

Sample Preparation for Analysis and Bioassay 1 g of the ground plant material was extracted with 5 ml of 80% MeOH (40–50 °C, 1 h × 2). The combined extract was concentrated *in vacuo*, and dried for use as the sample for the experiment.

Platelet Aggregation Measurement Platelets were obtained as platelet-rich plasma (PRP), which was prepared by centrifugation of fresh human (male) blood (1100 × g for 10 min) with a 1/10 volume of 3.8% sodium citrate aq. Platelet density was adjusted to 3.0 × 10⁵ per μl with platelet-poor plasma (PPP), which was obtained by centrifugation of the same blood at 3000 × g for 10 min. 200 μl of PRP was incubated with 25 μl of the test solution (80% MeOH extract was dissolved in distilled water or 2% dimethylsulfoxide (DMSO) solution and diluted to 1.0% with saline) at 37 °C for 4 min, then 25 μl of collagen (collagen reagent "HORM," Hormon Chemie Co., Ltd., Munich, Germany) was added to the reaction mixture as aggregating agent. It was confirmed that 2% DMSO in saline had no effect on platelet aggregation so this was used as the control. The aggregation profile was recorded in terms of the change of PRP light transmittance with constant stirring (5–7 min). Inhibition (percent) was calculated by the following equation.

$$\text{inhibition (\%)} = (1 - \text{sample value/control value}) \times 100$$

Quantitative Analysis of Ligustilide and Butylidenephthalide 80% MeOH extract prepared from 0.5 g of the ground material was extracted with 5 ml of CHCl₃ containing 50 μg/ml of naphthol as an internal standard (room temp., 30 min) and the upper layer after centrifugation (3000 × g, 10 min) was determined by GC. GC conditions-column: silicon OV-17 on Chromosorb W (AW-DMCS) (3 × 200 mm), column temperature: 200 °C, detector: FID, injector temperature: 250 °C, carrier gas: N₂ (30 ml/min), internal standard: α-naphthol.

Quantitative Analysis of Adenosine 0.5 g of the ground material was extracted with 5 ml of distilled water with continuous stirring (room temp., 40 min). 2 ml of MeOH containing 1.3 mg/ml of *p*-toluene sulfonic acid was added to 2 ml of the extracted solution as an internal standard and after centrifugation the supernatant was analyzed by HPLC. HPLC conditions-column: YMC A-303 (s-5 120A ODS) 4.6 × 250 mm, column temperature: 30 °C, detector: UV 254 nm, eluent: 5% CH₃CN/0.05 N phosphate buffer (pH 4.5), flow rate: 0.7 ml/min.

Results

Comparison of APA by Habitats Whether a wild or cultured material, the quality of a crude drug differs by habitat in many cases; on Touki cultured in various districts, a study was therefore made of the difference in APA effect. In this study Obuka Touki was employed, and all materials were treated with hot water to assure uniformly prepared samples. Meanwhile, in consideration of individual differences, samples were prepared using 3 to 5 stocks each from a respective habitat. Results indicated that even with the same species APA greatly differs by habitat (Table I).

TABLE I. Inhibitory Effect of *Angelicae Radix* (Obuka Touki) Cultivated in Different Districts on Collagen^{a)}-Induced Platelet Aggregation

Material ^{b)}	Yield of ext. (%)	Inhibition activity (%)		
		2×10^{-3}	1×10^{-3}	5×10^{-4} (g/ml) ^{c)}
1	36.4	86	75	31
2	33.1	38	13	—
3	39.2	63	25	—
4	29.9	79	48	15
5	35.5	88	86	70

a) At $1.0 \mu\text{g/ml}$ (final concentration). b) Material 1: commercial product (Tochimoto Tenkaido Co., Osaka), 2: Sibukawa, Gumma, 3: Fujimi-mura, Gumma, 4: Azuma-gun, Gumma, 5: Herbal Garden, Toyama Medical and Pharmaceutical University. c) Extract was dissolved in H_2O or 2% DMSO solution.

TABLE II. Inhibitory Effects of Morphologically Different Groups of Obuka Touki (I—III) and Hokkai Touki on Collagen^{a)}-Induced Platelet Aggregation

Group	Sampling No.	Yield of ext. (%)	Inhibition activity (%)	
			1×10^{-3}	5×10^{-4} (g/ml) ^{b)}
I	-1	36.8	35	—
	-2	33.6	35	—
	-3	43.5	31	—
	-4	34.2	28	—
	-5	39.4	32	—
II	-1	37.2	66	39
	-2	31.2	90	46
	-3	35.0	72	42
III	-1	44.5	46	—
	-2	39.1	32	—
	-3	39.8	48	26
Hokkai	-1	34.8	17	—
	-2	36.1	10	—
	-3	35.3	25	—
	-4	33.6	22	—

a) At $1.0 \mu\text{g/ml}$ (final concentration). b) Extract was dissolved in H_2O or 2% DMSO solution. Aspirin IC_{50} : 3.8×10^{-5} g/ml.

Relationship between Appearance and APA Even with the same species grown in the same district, the form differed largely, therefore, the morphological factor is one of the important aims for evaluation of the quality. Obuka Touki grown in the same location (Toyama Medicinal Plant Instructive Center) were divided morphologically into the following 3 groups to study their correlation with APA effects. Group I: Typical Obuka Touki showing glossy dark green leaves with many lobations, purplish stems and veins on the back of leaves. Group II: A slightly different morphologically from group I, showing very light purple stem color, with leaf veins being lighter green and not as glossy. Group III: Showing almost no characteristics of groups I and II: glossless leaves, shallow lobations, and strongly greenish as a whole. As shown in Table II, no correlation was observed in qualities determined morphologically from the above-ground part of Touki and those determined by their APA effects. In other words, group I, noted as good quality, did not show the APA effect as strongly as did group II, being rather uncharacteristic Obuka Touki. The same comparison was made using Hokkai Touki obtained from Nayoro, Hokkaido. Although yield of the extract was found to be nearly the same as that of Obuka Touki, the APA effect was markedly lower

Fig. 1. Relationship between Butyridenephthalide Content and APA Activity in Total Extract of *Angelica acutiloba* ($n=22$)

a) At 10^{-3} g/ml of extract.

Fig. 2. Relationship between Ligustilide Content and APA Activity in Total Extract of *Angelica acutiloba* ($n=22$)

a) At 10^{-3} g/ml of extract.

Fig. 3. Relationship between Adenosine Content and APA Activity in Total Extract of *Angelica acutiloba* ($n=22$)

a) At 10^{-3} g/ml of extract.

(Table II).

Relationship between APA and Ingredients The APA effect of Touki has been reported in the past,^{9,10)} but only adenosine¹⁰⁾ in the water soluble fraction is known as an active material. Thus, for the purpose of clarifying the components that support the APA effect of total extracts other than adenosine, ligustilide and butyridenephthalide, considered to be the major components of Touki, were first studied for their content in extracts and APA effect in each sample. Next, to determine whether or not adenosine can be a guide for quality evaluation of Touki, adenosine content in each sample and its correlation with the APA

effect were examined in the same manner. No clear correlation was noted between butyridenephthalide content in any extract and the APA effect (correlation coefficient: 0.5) (Fig. 1). On the other hand, a certain correlation was noted between ligustilide content and the APA effect (correlation coefficient: 0.7) (Fig. 2). Touki, which contains much phthalide, was not however always strong in the APA effect, making it rather difficult to evaluate the quality with this component. No better correlation was noted between adenosine, which itself shows a strong APA effect, content and APA (correlation coefficient: 0.5); thus it is not sufficient to evaluate the quality of Touki with adenosine only, because a sample containing about 0.03% of adenosine indicated a larger fluctuation in APA effect (Fig. 3).

Discussion

Within the same species of Touki, the APA effect has been suggested to be quite different by habitat. Conventional morphological evaluation as to the quality of Touki by its appearance is not always the same as that of the APA effect shown by Touki extract, and the samples assumed to be of better quality (group I in Table II) showed lower APA effect. Further, studies on ligustilide, butyridenephthalide, and adenosine isolated as active material in APA, which are considered to be used as indices for quality evaluation, as to their contents related to their APA effects, revealed no superior correlation that enables to obtain knowledge of endorsing the APA effect of Touki. APA active components other than adenosine have been studied, and the results will be reported in detail elsewhere, but it is concluded that the APA effect of Touki results from the combined action of plural components. That is, when the quality of Touki is evaluated in view of APA, estimation is very difficult if

based solely on the content of a specific component. The quality of a crude drug is conventionally evaluated by certain of its specific components; however, the result of this study indicated the importance of making this determination based on the action of total extracts combined to assure that the purpose of use is met. Also important is that the efficacy of a natural drug, which is often not scientifically explainable, be more seriously studied.

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References

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- 2) S. Tanaka, T. Hoshino, Y. Ikeshiro, M. Tabata and M. Konoshima, *Yakugaku Zasshi*, **97**, 14 (1977).
- 3) S. Takahashi, H. Hikino and Y. Sasaki, *Yakugaku Zasshi*, **78**, 1156 (1958).
- 4) H. Hikino, Y. Hikino, O. Tamura and S. Takahashi, *Syoyakugaku Zasshi*, **16**, 12 (1962).
- 5) T. Watanabe and M. Goto, *Yakugaku Zasshi*, **73**, 841 (1953).
- 6) T. Yamagishi, H. Kaneshima, Y. Kinoshita and M. Mori, *Hokkaido Institute of Public Health Report*, **24**, 47 (1974).
- 7) M. Goto, *Yakugaku Zasshi*, **75**, 1180 (1955).
- 8) K. Terasawa, A. Imadaya, H. Tosa, T. Mitsuma, K. Torizuka, K. Takeda, M. Mikage, M. Hattori and T. Namba, *Fitoterapia*, **LVI**, 201 (1985).
- 9) Z.-Z. Yin, *Yao Hsueh Hsueh Pao*, **6**, 321 (1980).
- 10) K. Torizuka, P. Nishiyama, I. Adachi, N. Kawashiri, M. Ueno, K. Terasawa and I. Horikoshi, *Chem. Pharm. Bull.*, **34**, 5011 (1986).